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EFFECTS OF LSD ON HUMAN CHROMOSOMES

RAZIA S. MUNEER

Department of Biochemistry and Molecular Biology, The University of Oklahoma Health Sciences Center, Oklahoma City, Okla. 73190 and Department of Pediatrics, Division of Medical Genetics, Washington University School of Medicine, St. Louis, Mo. 63110 (U.S.A.)

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Summary

A number of positive and negative studies have been reported with regard to the damaging effects of LSD on human chromosomes. The present report describes a comparative study of cytogenetic analyses of 200 metaphases of lymphocytes from each of 6 subjects (3 males, 3 females) at varying concentrations of LSD, along with a positive control with mitomycin C and a negative control with sterile water. Results of a small pilot study on the effects of LSD on growth, macromolecular synthesis, mutation, and recombination in bacteria, λ phage and mammalian cells are also included. The data failed to show any significant differences between chromosome aberrations and LSD. Significant changes in somatic cells and in chromosomes occurred only at high doses of LSD.

Introduction

In 1967, Cohen et al. [2,3] first demonstrated an increased chromosomal aberration rate in human lymphocytes treated in vitro with various concentrations of lysergic acid diethylamide-25 (LSD). Since that time, a series of in vitro and in vivo studies concerning its damaging effects have been published which were either conflicting or inconclusive. Recent reviews by Dishotsky and co-workers [6], Long [10] and Titus [14] have summarized and compared the studies dealing with chromosomal damaging, teratogenic and carcinogenic effects of LSD in vitro and in vivo.

Most of the chromosomal studies in vitro and in vivo were carried out on

Abbreviations: GCM, Gibco chromosome medium; LSD, lysergic acid diethylamide-25; MEM, Eagle's minimum essential medium; MMC, mitomycin C; PHA, phytohemagglutinin.

peripheral blood samples which were stimulated to divide in culture in the presence of phytohemagglutinin (PHA). In the case of the in vitro studies, lymphocytes were exposed to LSD during the process of cell division and the in vivo studies used lymphocytes of patients previously exposed to LSD.

In attempting to explain the discrepancy between the large effects reported for in vitro exposure and the smaller negative effects in vivo, a hypothesis was formulated that LSD induces chromosome aberrations only in dividing cells and has no effect on resting cells. This hypothesis could explain the aberration controversy. To test this hypothesis, a pilot study was carried out with growing mouse-L cells and human fibroblasts in culture exposed to LSD concentrations up to 100 μg/ml. While 100 μg/ml of LSD was toxic to growing cells, concentrations up to 10 μg/ml produced no inhibition of growth or macromolecular synthesis and no noticeable chromosomal aberrations. These negative findings raised the possibility that the great sensitivity of dividing cells might be cell-specific and limited to lymphocytes.

This led me to carry out an in vitro study on chromosome preparations from PHA-stimulated lymphocytes. Because the results differ from those of Cohen and co-workers [2,3], they cast doubt on the chromosomal-breaking potential of LSD at doses to which human cells are exposed.

The present study was designed with two types of controls: a negative control with no drug treatment, and a positive control using mitomycin C (MMC) which has been shown to cause significant single and double breaks in chromosomes [4]. LSD 25 and LSD tartrate were tested with two types of culture media and with two different periods of drug exposure.

Materials and methods

Blood was obtained from 6 normal healthy individuals (3 males and 3 females), who had not been previously exposed to LSD. Chromosomal preparations were made from 72-h cultures of heparinized blood lymphocytes stimulated with PHA. The culture media used were (a) Eagle's minimum essential medium (MEM) and (b) Gibco chromosome medium (GCM), both were supplemented with 20% fetal calf serum, 2% L-glutamine and 1% penicillin–streptomycin mixture. Cultures were harvested according to the method of Moorhead et al. [11]. LSD-25 or LSD tartrate was added to a replicate of each culture in concentrations of 1.0 μg/ml and 10 μg/ml final concentration at 24 and 72 h prior to harvest. An equal volume of sterile distilled water was added to the negative control cultures. Mitomycin C was added to the positive control cultures at a final concentration of 1 μg/ml in the case of LSD-25 series and 0.1 μg/ml in the case of LSD tartrate series with same exposure time. The dose was reduced to 0.1 μg/ml because 1 μg/ml MMC was lethal to cells. All slides were coded from a table of random numbers to prevent the observer from knowing the origin of the slide under analysis. Cells for analysis were selected under low power and co-ordinates for each suitable metaphase recorded. Complete numerical and morphological analysis for each metaphase was performed. At least 20—25 cells were counted for the modal chromosome number. 100 suitable metaphases from each treatment for each subject were studied for aberrations. If 100 metaphases were not available, whatever number was analyzable was included.

The following structural aberrations were scored:

(1) *Gap*: a discontinuity of chromatin material in a chromatid without displacement of the distal portion. All gaps were scored but were not included as aberrations in calculating the total frequency of chromosome aberration in conformity with Revell [12].

(2) *Chromatid break*: a discontinuity of chromatin material in only one chromatid of a chromosome with displacement of the broken segment.

(3) *Isochromatid break*: as in (2) but both sister chromatids broken at the same level with displacement of the broken segments.

(4) *Fragment*: dislocated centric or acentric portions of chromosomes arising as a result of a chromosomal structural change.

(5) *Dicentric*: functionally single chromosome with two centromeres formed by fusion of portions of two chromosomes.

(6) *Quadriradial*: translocated combination of two or more chromosomes often forming quadriradial figures.

(7) *Ring*: a physically circular chromosome, single or chain-linked depending on the number of chromatid ends joined with each other.

Results

Cytogenetic findings regarding the effect of LSD-25 on lymphocytes are shown in Table 1. This table represents cumulative data on the cells of 6 subjects treated for 24 and 72 h with 1 μg/ml and 10 μg/ml LSD-25 in the two different media. Gaps were the most common morphological variation observed in both treated and non-treated cells. The control frequency of metaphases

TABLE 1

COMPARISON OF THE NUMBER OF CHROMOSOME ABERRATIONS IN HUMAN LYMPHOCYTES TREATED WITH LSD-25

Treatment		Control	LSD		MMC 1 μg/ml
			1 μg/ml	10 μg/ml	
Number of cells scored		2400	2333	2323	335
Types of aberrations					
Gaps	Single	189	105	149	166
	Isochromatid	3	4	5	24
Breaks	Single	6	11	5	129
	Isochromatid	2	0	2	22
Fragments		7	13	12	51
Dicentrics		0	0	0	8
Quadriradials		0	0	0	91
Rings		0	0	0	2
Deletions		0	0	0	0
Total aberrations excluding gaps		15	24	19	303
Percent of aberrations excluding gaps		0.62	1.02	0.82	94.58
Probability			(X^2 = 2.35) P = 0.14	(X^2 = 0.610) P = 0.425	

TABLE 2

COMPARISON OF THE NUMBER OF CHROMOSOME ABERRATIONS IN HUMAN LYMPHOCYTES TREATED WITH LSD TARTRATE

Treatment		Control	LSD		MMC 0.1 μg/ml
			1 μg/ml	10 μg/ml	
Number of cells		973	1021	843	847
Types of aberrations					
Gaps	Single	62	86	58	161
	Isochromatid	3	2	3	4
Breaks	Single	7	4	5	33
	Isochromatid	3	1	3	0
Fragments		4	15	11	47
Dicentrics		0	0	0	18
Quadriradials		0	0	0	32
Rings		0	0	0	2
Deletions		0	1	0	0
Total aberrations excluding gaps		14	21	19	132
Percent of aberrations of excluding gaps		1.45	2.05	2.25	15.6
Probability			(X^2 = 1.07) P = 0.295	(X^2 = 1.64) P = 0.20	

with aberrations was 0.62%, whereas cells treated with 1 μg/ml LSD-25 had 1.02% cells with chromosome aberrations, and cells treated with 10 μg/ml LSD-25 had 0.82% aberrations. These differences were not significant.

Table 2 summarizes the results obtained with LSD tartrate on cells from 3 subjects. Although the frequency of fragments is slightly increased, neither fragments nor total aberrations are significantly increased. The slight increase with LSD was not dose-dependent. The aberration frequency in cells exposed to 0.1 μg/ml MMC was 10 times higher than the non-drug control.

The cumulative data presented in Tables 1 and 2 was analyzed with respect to the dosage and exposure time to LSD (Table 3). In this analysis, there was no significant difference between cells exposed to LSD-25 or LSD tartrate for 24 h and the controls. Similarly, cells exposed for 72 h to LSD-25 also revealed

TABLE 3

COMPARISON OF CHROMOSOME ABERRATIONS INDUCED BY VARIOUS DOSAGES AND EXPOSURE TIMES TO LSD

Exposure time	Control	LSD-25		MMC 1 μg/ml	Control	LSD tartrate		MMC 0.1 μg/ml
		1 μg/ml	10 μg/ml			1 μg/ml	10 μg/ml	
24 h	10/1200	11/1200	6/1112	303/335	12/463	13/469	9/455	33/458
Probability		0.85	0.41			0.625	0.53	
72 h	5/1200	13/1133	13/200	no cells	2/510	8/552	10/398 [a]	99/389
Probability		0.045	0.06			0.625	0.006	

[a] Significant at the P = <0.01 — at 0.025 rejection level.

no difference between the aberration frequency at both levels of 1 μg/ml and 10 μg/ml. However, cells treated with LSD-tartrate for 72 h showed a significantly higher aberration frequency compared to the controls ($X^2 = 7.5$, $P = 0.006$).

It is significant that complex aberrations such as dicentrics and quadriradials were not seen in these studies. Most of the aberrations observed represent the results of single break events. Statistically no significant differences were found among the 6 subjects with regard to sex or age. The modal chromosome number remained 46 in all scored cells.

In contrast to the studies with LSD, the exposure to MMC induced chromosomal aberrations. The frequencies of different types of aberrations were much greater than in control cells. With the concentration of 1 μg/ml MMC, exposed for 24 h, many cells were found to show multiple aberrations such as chromatid breaks, fragments, dicentrics and quadriradials. The aberration frequency remained impressive when the concentration was reduced 10-fold. In addition, the types of aberrations observed with MMC were severe and are not comparable to the aberrations observed in the case of LSD. This confirms the work of Cohen and Shaw [4].

Other studies

In an effort to get a simpler system to study LSD effects on nucleic acid metabolism, LSD-25 was also studied for its effects on bacteria, mouse-L-cells, and human fibroblasts. Using *E. coli* K_{12}, C600 and W3350, LSD-25 at concentrations of 100 μg/ml showed no effects on growth rate or viability. It also produced no curing of a F-gal episome in a K_{12} strain carrying this episome. Bacteriophage λc_{I7} was used to test for mutagenesis of LSD in culture. This phage acquires virulence with a second mutation in the c1 repressor gene. No increase over the background level of virulent mutants (1×10^{-4}) was produced by growth of phage infected bacteria in LSD at 100 μg/ml level. Finally, addition of LSD at this concentration did not modify the recombination frequency between the two amber mutants O sus_{29} and N sus_{53} in *E. coli* C600. Thus, all studies on *E. coli* were negative at this concentration. However, it is not certain that LSD gets into the bacterial cell under these circumstances. Work by Vann [16] did show a weak mutagenic effect of LSD at 10-fold or more higher concentrations.

Mouse L-cells were exposed to LSD-25 during growth in culture. A level of 10 μg/ml produced no effects on growth rate or rates of macromolecular synthesis. However, 100 μg/ml produced a marked inhibition (40—60%) in the increase in cell number, total protein synthesis and thymidine incorporation into DNA. Monolayer cells became swollen, granular and detached from the surface after 48 h.

Human fibroblasts growing in monolayer showed a similar insensitivity to low levels (10 μg/ml) and toxic responses to 100 μg/ml with comparable inhibition of growth and macromolecular synthesis and eventual cell death.

Discussion

The intent of this study was to test the hypothesis that the discrepancy between the predominantly positive in vitro studies demonstrating that the

LSD induces chromosome aberrations and the predominantly negative results from patients ingesting LSD could be explained by a difference in sensitivity between dividing and non-dividing cells. Failure to obtain any evidence for effects of doses up to 10 μg/ml on dividing cells led me to question whether dividing lymphocytes are sensitive to doses to which humans might be exposed.

Recent reviews [6,10,14] summarizing positive and negative studies also point out that in spite of positive findings no consistent dose relationship was found and positive results were reported only with dosages that far exceeded likely human exposure. This finding contrasts with those of Cohen et al. [2,3], Kato and Jarvik [9], and Corey et al. [5], who reported striking increases in the frequency of chromosome aberrations and a decrease in mitotic indices. Although the mitotic indices varied from 4.5 to 11.9% among the donors, there was no statistical difference between cultures exposed or not exposed to LSD.

The present study does not demonstrate the significantly increased breakage reported by others. Cohen and associates [2,3] reported a range of 7.7—17.5% breaks in the LSD-treated cultures as compared to an average of 3.9% breaks in control cultures. Similarly, Corey et al. [5] reported a range of 4—18.70% chromosomal breaks in the treated cells and a range of 0—15% breaks in the control cells. Kato and Jarvik [9] also studied the damaging effects of LSD with the addition of a comparison between the effects of LSD, ergonovine maleate, aspirin and streptonigrin. They found a breakage frequency range of 0—9% in control cells, and a range of 0—15.12% in treated cells. They concluded that LSD was as effective as the other tested chemicals in producing chromosomal aberrations. The present study was also designed along the lines of the original work by Cohen and co-workers [2,3] with slight modifications, and with the addition of MMC as a positive control. When the results are compared with the positive studies which are listed above, the frequency of breaks in control cells is lower (range 0.62—1.45%) and the range of breakage in treated cells is also lower (0.82—2.25%). However, these workers did not list each type of aberrations separately; instead they listed only the break frequency.

The aberration frequencies calculated individually either on the basis of the dose or the exposure time, did not differ significantly when the cells were treated with LSD-25, see Table 3. Whereas, with LSD-tartrate the aberration frequency was significant at 72-h exposure. It cannot be explained why the aberration frequency was high in this group. It could be attributed to the small size of the scorable cell population which made the frequency statistically more significant. Or it could have resulted from an unusually low aberration frequency in the control for those samples rather than from an increase in the aberration frequency of cells exposed to LSD-tartrate. In view of the large number of comparisons available one P value at about the 5% level is not unexpected by change alone.

There have been a number of other negative studies which do not confirm the chromosome-breaking effect of LSD. Sturelid and Kihlman [13] tested the effects of LSD on chromosomes in three different types of cells, viz. root-tips of *Vicia faba*, Chinese hamster cells and human lymphocytes. Since LSD did not produce any chromosome abnormalities, they concluded that LSD is not a chromosome-breaking agent in these test systems. Recently Dorrance and associates [7] also failed to show any evidence of chromosomal damage in LSD-

exposed lymphocytes and fibroblasts. Amarose et al. [1] found no relationship between LSD and chromosome damage using rabbits as testing systems. Similar to these findings, Dowler and Wolpert [8] did not find any effect of LSD on *E. coli* B/Rλ growth at a concentration of 100 μg/ml but did find reversible growth inhibition at much higher concentrations. Even the largest controlled in vivo study conducted by Tjio et al. [15] involving 32 subjects concluded that there was no significant amount of chromosomal damage before or after exposure to LSD.

This study on peripheral lymphocytes permits no conclusions concerning possible mutagenic or other deleterious effects of LSD on human somatic cells at reasonable doses. Clearly LSD at concentrations of 100 μg/ml is toxic to growing mammalian cells, and appropriate test systems might implicate an effect of LSD on replication or expression of genetic material in this toxic effect.

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